F IRI/T164

FAO Fisheries Technical Paper No. 164 FIRI/T164

SEP 29 1977

MANUAL OF METHODS IN AQUATIC ENVIRONMENT RESEARCH

Part 4 – Bases for Selecting Biological Tests to Evaluate Marine Pollution

with the cooperation of the
United Nations Environment Programme

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

UNITED NATIONS ENVIRONMENT PROGRAMME

MANUAL OF METHODS IN AQUATIC ENVIRONMENT RESEARCH

Part 4 - Bases for Selecting Biological Tests to Evaluate Marine Pollution

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, May 1977

The designations employed and the presentation of material in this publication do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

M-45

ISBN 92-5-100300-9

The copyright in this book is vested in the Food and Agriculture Organization of the United Nations. The book may not be reproduced, in whole or in part, by any method or process, without written permission from the copyright holder. Applications for such permission, with a statement of the purpose and extent of the reproduction desired, should be addressed to the Director, Publications Division, Food and Agriculture Organization of the United Nations, Via delle Terme di Caracalla, 00100 Rome, Italy.

©FAO 1977

PREPARATION OF THIS MANUAL

This Manual was prepared as part of a cooperative project of the United Nations Environment Programme entitled

Effects of Pollutants on Living Aquatic Resources and Scientific Basis for Monitoring

with the Food and Agriculture Organization of the United Nations as cooperating agency.

Distribution

FAO Department of Fisheries
FAO Regional Fishery Officers
Selector SM
ACMRR Members
UNEP

Bibliographic Reference

FAO (1977)
FAO Fish.Tech.Pap., (164):31 p.
Manual of methods in aquatic environment research. Part 4. Bases for selecting biological tests to evaluate marine pollution

FAO manuals. Water pollution. Bioassays. Toxicity tests. Water quality. Pollution monitoring.

CONTRIBUTORS
(Members of the ACMRR/IABO Working Party on Biological Effects of Pollutants)

P.A. BUTLER
EPA, Gulf Breeze Laboratory, Sabine Island, Gulf Breeze, Florida 32561, U.S.A.

P. DOUDOROFF
Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon 98331, U.S.A.

M.A. FONTAINE[3/]
Museum national d'histoire naturelle, rue Cuvier 7, Paris V, France

M. FUJIYA
Water Quality and Fish Pathology Research Division, Freshwater Fisheries Research Laboratory, 339 Miya Hinoshi, Tokyo, Japan

R. LANGE[1/]
Institute of Biology, Odense University, Niels Bohrs Alle, DK5000 Odense, Denmark

R. LLOYD
Salmon and Freshwater Fisheries Laboratory of the Ministry of Agriculture, Fisheries and Food, 10 Whitehall Place, London SW1, U.K.

D.J. REISH[2/]
Department of Biology, California State University, Long Beach, California 90804, U.S.A.

J.B. SPRAGUE[1/]
Department of Zoology, University of Guelph, Guelph, Ontario, Canada

M. SWEDMARK
Kristineberg Marine Biological Station, 45034 Fiskebäckskil, Sweden

---

1/ Attended First Session only
2/ Attended Second Session only
3/ Contributed through correspondance

PREFACE

At its Seventh Session in October 1973, the FAO Advisory Committee on Marine Resources Research (ACMRR) agreed to establish, jointly with the International Association for Biological Oceanography (IABO), a Working Party whose task would be "... to review and evaluate critically the methods for bioassays and toxicity tests presently used ..." and, specifically, "... to advise on the practical use of tests ...", and "... to identify research programmes required to improve methodology and their application ...".

Under the cooperative project of the United Nations Environment Programme (UNEP) entitled "Effects of Pollutants on Living Aquatic Resources and Scientific Basis for Monitoring", the Working Party held two sessions (FAO Fish.Rep. 187). The first session was held at FAO Headquarters, Rome, from 27 to 31 October 1975. The second session was held at the Inter-University Centre for Post-graduate Studies, Dubrovnik, 22-25 November 1976, preceded by an Expert Consultation on Bioassays with Aquatic Organisms in Relation to Pollution Problems, 15-19 November (FAO Fish.Rep. 187).

This Manual is the end product of the two sessions of the Working Party; proposals and recommendations received from the Expert Consultation were taken into account and are reflected herein. The Manual has been produced as a corporate effort and is intended as a quick means for selecting suitable biological test methods for evaluating marine pollution.

The first session of the Working Party was chaired by Dr. J.B. Sprague, of the Department of Zoology of the University of Guelph, Ontario, Canada. In the absence of Dr. Sprague at the second session, the chair was taken by Dr. P.A. Butler, from the EPA Gulf Breeze Laboratory, Florida, U.S.A. Final editing was carried out by the staff of the Fishery Resources and Environment Division of FAO, particularly Dr. H. Naeve, who, together with Mr. A. Wenblad, also acted as Technical Secretary of the Working Party.

The views expressed in the Manual are those of the Working Party and do not necessarily represent the views of either FAO or UNEP.

CONTENTS

		Page
1.	INTRODUCTION	1
	1.1 Scope of the Manual	1
	1.2 Biological and Chemical Approaches to Discharge Regulations	2
	1.3 Other Working Parties with Related Terms of Reference	2
2.	SOURCES AND EFFECTS OF MARINE POLLUTION	2
	2.1 Sources	2
	2.1.1 Fixed-point sources	2
	2.1.2 Variable location/random-point sources	3
	2.1.3 Diffuse sources	3
	2.2 Effects	3
3.	PURPOSE OF TESTS	3
	3.1 Screening Tests	3
	3.2 Regulatory Tests	4
	3.3 Tests to Establish Water Quality Criteria	4
	3.4 Effluent Monitoring Tests	4
	3.5 Tests for Monitoring Discharge Areas	4
	3.6 Tests to Protect Higher Trophic Levels	4
	3.7 Organoleptic Tests	4
	3.8 Tests for Biostimulation	4
4.	TYPES OF TEST PROCEDURES	5
	4.1 Direct Response Tests	5
	4.1.1 Toxicity tests	5
	4.1.2 Biostimulation tests	6
	4.1.3 Repellence bioassay	6
	4.1.4 Tests for bioaccumulation and trophic accumulation	6
	4.1.5 Ecological surveys	7
	4.2 Indirect Response Tests	7
	4.2.1 Organoleptic tests	7
	4.2.2 Ecological survey	7
	4.2.3 Biostimulation tests	8
	4.3 Recommended References for Test Procedures	8
	4.4 Types of Procedures to be Used for Specific Purposes	9
5.	SPECIFIC USES OF THE VARIOUS TEST PROCEDURES FOR POLLUTION IDENTIFICATION, EVALUATION AND CONTROL	9
	5.1 Fixed-point Discharge	12
	5.2 Variable location/Random-point Discharge	12
	5.3 Diffuse Pollution	13
6.	SELECTION OF TEST ORGANISMS	13
	6.1 General Considerations	13
	6.2 Organisms for Screening and Regulatory Tests	13
	6.3 Organisms for Other Bioassays	14
	6.4 Special Characteristics of Some Test Groups	15
	6.5 Selected Bibliography	15

		Page
7.	SALINITY OF TEST SOLUTIONS	16
8.	INTERPRETATION OF RESULTS	17
	8.1 General Aspects	17
	8.2 Ecological Significance of Test Results	17
	8.3 Measurement of Toxicity	18
	8.4 Application Factors	19
	8.5 Concentration Versus Amount of Toxicants	19
9.	REFERENCES	19
Appendix 1:	Examples of Investigational Programmes	23
Appendix 2:	Calculation and Use of the Toxicity Emission Rate	27
Appendix 3:	Identification of Research Needs	30

1. INTRODUCTION

It is becoming increasingly obvious that oceans do not provide limitless dilution for man's waste products. Coastal and estuarine areas are particularly sensitive to pollution because dilution available for waste disposal may be small, or even irrelevant for certain kinds of pollutants, because they are vital for commercial fisheries, and because they are becoming increasingly important for mariculture. Pollution of the oceans may cause widespread problems which only international action could resolve.

Prevention of pollution of the sea is primarily aimed at protecting both public health and living organisms, especially fish and shellfish. Public health aspects of marine pollution are not within the terms of reference of this manual. The most direct method of evaluating the effects of marine pollution on organisms is by ecological studies reinforced where necessary by the methods considered in this manual: bioassays and other methods of bioevaluation.

However, it must be remembered that overall evaluation of marine pollution requires simultaneous consideration of other aspects, such as monitoring programmes for pollutants in the seas, chemical examination of waste components, and fate of materials in marine systems.

There is less experience with pollution control in marine waters than in freshwater rivers and lakes. However, many of the methods used to evaluate pollution in freshwater are entirely appropriate for marine work. This is especially true of bioassays, in which the basic techniques are the same with either kind of water.

1.1 Scope of the Manual

This manual is intended to offer help to beginners, in one particular field: selecting appropriate methods for bioevaluation of marine pollutants. Such a rationale is largely lacking in the literature, which abounds with detailed descriptions of test methods, but provides little guidance on choice of methods for attacking particular problems. This manual aims to partially fill this gap, a task which is considered to be the most useful one that could be undertaken at this time, within the terms of reference.

The emphasis of this manual is placed on the evaluation of the effects of chemical pollutants on marine organisms. The evaluation of effects of organic wastes, which cause a depletion of the dissolved oxygen content of the water, is a complex problem and is not given detailed consideration here. For this reason, the biochemical oxygen demand (BOD) test is not covered in this manual.

In this manual separate consideration is given to a number of topics which provide a framework for setting up a programme for biological evaluation of a particular chemical pollutant or complex industrial waste. The topics are described in the following sectional order:

(i) Sources of marine pollution, which section categorizes the different routes and time spans of the entry of pollutants into the marine environment, since these can require different methods for their bioevaluation.

(ii) Major purposes of tests, which section outlines the functions of different types of bioevaluation.

(iii) Types of procedures, which section briefly describes the basic principles of bioevaluation tests which have been developed; a selected bibliography of standard works which include detailed

descriptions of these types of procedures is included in this section. An attempt is made to interrelate components of topics (i)-(iii) into combinations which guide the investigator in selecting certain kinds of tests.

(iv) Types of test organisms, considered most appropriate for marine pollution evaluation.

Sections on salinity of test solutions and interpretation of test data are also included, since such general advice is scarce and scattered in the literature.

1.2 Biological and Chemical Approaches to Discharge Regulations

In regulating discharges of wastes toxic to aquatic life, data from toxicity tests with living organisms should be used and relied upon in addition to detailed studies on the physical and chemical characteristics of the pollutants and the changes which occur after discharge to the sea. Where the toxic components of a pollutant can be accurately described in chemical and physical terms, and relevant analytical techniques are available, and where sufficient is known of the toxicity of the components to aquatic life, then standards for that pollutant can be set as numerical values for these components. However, where the toxic components are unknown, or cannot be adequately analysed for, or where there is a discrepancy between the toxicity predicted from chemical data and the results of a bioassay, toxicity testing techniques with living organisms should be used and relied upon in preference to chemical standards. In this case, criteria or standards prescribing minimum acceptable dilutions or concentrations should be expressed in biological terms, such as the maximal fraction of the lethal concentration for a suitable test animal. It must be stressed, however, that a close collaboration between biologists and chemists should be maintained throughout an investigational programme.

1.3 Other Working Parties with Related Terms of Reference

There is a parallel ACMRR/IABO Working Party on Ecological Indices of Stress to Aquatic Systems (ACMRR/IABO Working Party, 1976). A Working Party on Biological Accumulators is also sponsored by ACMRR (ACMRR Working Party, 1975, 1975a). Several reports have been prepared by working groups on the scientific bases for the determination of concentrations and effects of marine pollutants, including Principles for Developing Coastal Water Quality Criteria (IMCO/FAO/Unesco/WHO/WMO/IAEA/UN, 1975). In accord with the Oslo Convention, ICES has set up a programme to monitor ocean pollution, with some bioevaluation included.

Beyond this, several groups have been working to standardize methodology, and these are referred to in our recommendations on methods. International organizations which have an interest in this field are the International Standards Organization (ISO) and the World Health Organization (WHO).

2. SOURCES AND EFFECTS OF MARINE POLLUTION

2.1 Sources

These can be categorized on the basis of either their temporal or spatial occurrence. Because the intention of this manual is to assist those responsible for assessing and managing discharges to the marine environment by use of toxicity tests and bioevaluation, a spatial differentiation is more appropriate with temporal subdivisions.

2.1.1 Fixed-point sources

Continuous discharges include: outfalls from municipal or industrial sewers containing organic, inorganic or thermic wastes; anti-fouling chemicals leaching from fixed or stationary structures in harbours.

Intermittent discharges include: bilge-pumping into harbours; anti-fouling chemicals from ships on fixed mooring; storm-water sewers and drainage ditches, and intermittent industrial discharge through pipelines.

2.1.2 Variable location/random-point sources

Continuous or nearly continuous discharges include very frequent sludge dumping at a particular location. The location could, of course, change from time to time.

Intermittent discharges include: occasional sludge, industrial waste, or dredged soil dumping, oil slicks or their treatment by dispersants or emulsifiers; accidental discharge and bilge-pumping.

2.1.3 Diffuse sources

Diffuse sources include river discharges of natural or man-made pollutants, run-off from agricultural land and atmospheric fallout of organic biocides.

2.2 Effects

The pollutants emanating from the sources listed above may be divided into two main groups. Those of one group have well-defined, direct and undesirable effects on populations of marine organisms. This group includes heat and toxic chemical pollutants which may be readily degraded, such as phenol or persistent and possibly bioaccumulative toxicants such as organochlorine pesticides. Those of the other group do not have the above effects but modify the environment in a way that undesirably affects the biota. This group includes non-toxic organic or inorganic solid materials which may remain in suspension interfering with light penetration and with algae photosynthesis, or settle out, affecting the benthic biota, and effluents with a high biochemical oxygen demand which give rise to low levels of dissolved oxygen. The effects of these pollutants are termed indirect.

3. PURPOSES OF TESTS

This section outlines the various purposes for which tests are undertaken. In order to protect the marine environment, upper limits have to be set for the discharge of harmful chemical and physical pollutants, and the subsequent discharge has to be monitored and regulated. The upper limits for discharges are derived from a consideration of the appropriate water quality criteria developed from response data for biological systems. The response measured may be lethal or sub-lethal, and the systems can range from ecosystems to enzyme reactions. Similarly, monitoring and regulatory tests can make use of a variety of responses, and the world literature abounds with descriptions of a multitude of test procedures. However, these tests can be broadly categorized into distinct groups, as shown in this section and the following section.

3.1 Screening Tests

Screening tests are done to obtain approximate indications of the concentrations of substances, singly or in mixtures, which are likely to be hazardous to marine life. Results are used as an approximate guide to the risk involved with continuous discharge, or a single occurrence. They can also be used to provide a ranking of substances in order of their toxicity; for example, to aid in the choice of oil spill dispersants.

Screening tests should utilize a few "standard" test species so that data obtained in different regions are comparable. The complete test procedure can be standardized if there is a real need for a widespread use of a common methodology. The tests are generally single-sample bioassays of simple design, and they usually determine acutely lethal effects,

although sub-lethal effects can be used. Screening tests give only a rough guide to ecological effects following the discharge of a pollutant, but they may provide sufficient information for an assessment of the degree of risk incurred by the discharge of the pollutant.

3.2 Regulatory Tests

Regulatory tests, or legal tests, may be considered as types of screening tests, used to decide on whether a specific discharge passes or fails some regulation or law. Rigid methodology is usually prescribed.

3.3 Tests to Establish Water Quality Criteria

Tests to establish water quality criteria are used to give fuller and more accurate information on the degree of risk of different types of pollution, as a base for the accurate prediction of "safe" levels without ecological consequences. These are non-standard tests which include a wide variety of research methods with many species and can measure the effects of chronic or sub-lethal exposures. They are preferably checked against ecological studies although such verification is difficult, since in nature there is usually no control of concentrations or other factors. Small controlled-ecosystem experiments may provide useful supporting information.

3.4 Effluent Monitoring Tests

Effluent monitoring tests are carried out to monitor discharges and catch unexpected peaks of toxicity. They are especially useful for complex mixtures which are difficult to analyse chemically. Since the pollution situation is local and may be unique, the tests are not necessarily standardized. They may be on-line tests for lethal or sub-lethal effects, and may be combined with an early-warning system.

3.5 Tests for Monitoring Discharge Areas

Tests for monitoring discharge areas are carried out to provide continuous surveillance of a waterbody. Action may then be taken if the water quality becomes unsuitable for marine organisms. These are *in situ* tests, usually for lethal or sub-lethal effects on animals in cages. They may be combined with ecological surveys to detect long-term effects.

3.6 Tests to Protect Higher Trophic Levels

Tests to protect higher trophic levels are designed to identify substances that accumulate within the tissues of a marine organism and harm other animals that eat the marine organism. Data from such tests can be used for the setting of standards to protect public health.

3.7 Organoleptic Tests

Organoleptic tests are done to determine the acceptability of a marine product for human consumption.

3.8 Tests for Biostimulation

Tests for biostimulation have the purpose of identifying problems of accelerated eutrophication. Either laboratory tests or field studies may be used to assess stimulation of algal growth.

4. TYPES OF TEST PROCEDURES

This section lists, with some explanation, the basic principles of the tests which are available. Whereas the foregoing section 3 dealt with the reason for doing the tests, this present section deals with the choices available in design and mechanics of test procedures. There is some overlap between the two sections, since the type of test often coincides with the reason for doing it.

In keeping with the purpose of presenting an overall view, there will be no attempt to give details of methods. Instead, the reader is referred to some of the good publications on methodology which already exist.

The word bioassay is used in this manual according to the following definition:

> "Bioassay signifies a test in which a living tissue, organism or group of organisms is used as a reagent for the determination of the potency of any physiologically active substance of unknown activity."

Thus, it is appropriate to speak of a toxicity bioassay or repellence bioassay of an industrial effluent or of some chemical of unknown toxicity. "Bioassay" has different usages in different countries. Sometimes these usages are wide, embracing almost any kind of test with organisms, but there is benefit in adhering fairly closely to the English dictionary meaning.

4.1 Direct Response Tests

4.1.1 Toxicity tests

Single-sample toxicity bioassay. This is any bioassay performed on a chemical or on a single "grab" sample of a complex waste. This single sample is used to make up different dilutions which are tested in the bioassay. The objective is to determine the concentration which is just sufficient to produce, after a given time exposure (short, long, or indefinitely continued), a certain response indicative of toxicity, such as death, immobilization, loss of equilibrium, impairment of reproduction, growth, or swimming ability, histological or biochemical changes, etc. The test may also be designed to determine the speed or degree of response to known, appropriate concentrations of a chemical or waste. The duration of a single-sample bioassay of a wastewater often cannot be very long because the sample cannot be stored for a long period without excessive change of its potency, usually a loss. Single-sample toxicity bioassays can be static or constant-flow.

A static bioassay is performed without continuous, constant-flow renewal of dilutions tested, but with or without their periodic renewal. Such renewal may be necessary when important toxicants deteriorate or are absorbed or otherwise lost rapidly enough to influence the test results markedly. Static toxicity bioassays may be superior to continuous-flow tests when highly toxic materials are gradually produced or released from relatively harmless waste components, such as settleable solids, sediments, or chemically unstable compounds. In such cases, aging of the test medium is necessary for detection and measurement of its latent toxicity. Such tests can be particularly useful in the evaluation of the effects of a sporadic discharge or accidental spill, where organisms are exposed to initially high concentrations which then decline because of chemical instability of the pollutant.

A constant-flow bioassay is performed with continuous or nearly continuous renewal of dilutions tested, so as to maintain nearly constant concentrations of active toxicants. This type of test is also called "flow-through" or "continuous-flow".

On-line effluent toxicity bioassay. This is a bioassay performed to determine, for an effluent of constant or variable toxicity, the concentration just sufficient to produce

after a given exposure, a certain response of test organisms that is indicative of toxicity, such as death, immobilization, etc. The effluent is continuously drawn off from a discharge line or conduit tapped at a suitable point, so the test dilutions are continuously replaced with dilutions of the effluent similar to that which is being currently discharged. In such a bioassay, dilution ratios remain unchanged, but the toxicity of each tested dilution will fluctuate throughout the test in accordance with fluctuations in quality of the effluent.

On-line early-warning effluent toxicity test. This is designed to monitor an effluent and give warning of an increase in toxicity. It is a continuous test of the undiluted or suitably-diluted effluent for its ability to elicit some appropriate, rapidly-pronounced and readily detected response of test organisms. In such a test, the medium is continuously renewed as in an on-line effluent toxicity bioassay, but usually only one concentration is tested.

In situ receiving water toxicity test. This test is performed in the field to determine whether water that has received wastes or other pollutants produces some measurable response, usually death of test organisms. Organisms are experimentally exposed to the water in cages or live-cars, or in aquaria through which the water is pumped. Such tests can be performed at various distances from a waste outfall, where the waste has been naturally diluted and aged in varying degrees.

4.1.2 Biostimulation test

Such a bioassay with algae is very similar in method to single-sample toxicity bioassays, but has a different purpose. It detects and measures the ability of wastes or chemicals to stimulate multiplication and growth, an effect (eutrophication) that often results in an over-abundance or "bloom" of algae, with adverse effects on beneficial uses of the receiving waters.

4.1.3 Repellence bioassay

This test attempts to measure, in the laboratory, avoidance reactions of marine animals to a pollutant. The organism, usually a fish or a large crustacean, is given a choice between "polluted" and "clean" waters in a small tube or tank; the gradient at the interface may be steep. The same apparatus and procedures will usually also measure attraction to the pollutant, if any exists. Attraction to an effluent and continued residence in it may lead to deleterious effects from chronic exposure.

For motile species, avoidance may sometimes be the key sub-lethal response, more sensitive and more significant in nature than impairment of reproduction measured by chronic toxicity tests (e.g. Geckler, in press). However, it is particularly difficult to predict from such laboratory results what would happen in the field (see section 6). The topic is put in a separate category from direct toxicity tests because the avoidance response may or may not be related to toxicity of the pollutant. In some cases, organisms may not avoid, or may be attracted to, particular toxic concentrations of a waste (e.g. Sprague and Drury, 1969). Other behavioural responses, and effects through damage to sensory receptors, are included under direct responses.

4.1.4 Tests for bioaccumulation and trophic accumulation

Such tests are required for materials which accumulate in marine biota; high tissue concentrations of these substances may cause death, but lower amounts may be accumulated over a period of time without harming the organism. In the latter case, predators may accumulate the compound to an extent which is harmful to them or to the predators at the next trophic level. Concentrations in water should be correlated not only with responses of the organisms, but also with measurements of the accumulated pollutants in the tissues. This is because it is easier subsequently to monitor the levels in a specific organism

than those in the aqueous medium. Examples are copper and other heavy metals which accumulate in shellfish, and organochlorine pesticides which can accumulate in most marine animals and fish-eating birds.

This topic is covered by a special working party and the reader is referred to the manual produced by it for approaches and detailed procedures (Portmann, 1976). Here it is only necessary to note that effects may be assessed by field studies or by specially designed laboratory experiments.

4.1.5 Ecological surveys

This topic is also the subject of a special working party (ACMRR/IABO Working Party, 1976). It must be stressed that field observations are the ultimate bioevaluation, to ascertain whether laboratory tests for lethal and sub-lethal toxicity can in fact be predictive of what happens in nature. Ecological surveys are also the ultimate procedure for monitoring effectiveness of waste control. Natural communities are day-long, year-around, integrating monitors of the degree of pollution. Thus, ecological surveys are usually more instructive than chemical monitoring. Nevertheless, it is usually beneficial to have parallel physico-chemical assessment of pollution to correlate harmful effects with concentrations in particular locations. This is especially helpful in assessing the relative importance of pollutants from two or more sources, which affect the same area.

The investigation or survey usually requires a detailed analysis of the epibenthic and benthic community with regard to number of species, population density of each species, and apparent well-being of the community compared with one in a nearby similar habitat in which pollution is not suspected. It is important to recognize that pollution may cause a shift in community composition or change in species diversity, but not necessarily a change in population density. A well-defined and unexpected change in the community composition, regardless of the number of species involved, suggests a pollution factor and warrants a more detailed investigation of possible toxic inputs into the area. At the research level, overall ecological assessment of pollutants may be done by creating small artificial ecosystems (Hansen, 1974), or by manipulating small natural ones which are useful, but time-consuming and complex, methods of bioevaluation.

4.2 Indirect Response Tests

4.2.1 Organoleptic tests

Some pollutants may produce unpleasant taste or smell in harvestable aquatic organisms such as fish, crustaceans or molluscs. The pollutant may not harm the marine organism, but the fishery resource may be spoiled as an economic asset. In assessment of this problem, human "tasters" are used as the bioassay organisms. The topic is complex and difficult to assess accurately, and should not be attempted by amateurs. The best procedure is to involve those experienced in food science, using large numbers of trained tasters, and adequate controls.

4.2.2 Ecological survey

The same general principles apply here as were outlined above. Indirect effects include those of suspended and settling solids or deoxygenating wastes which do not have a direct toxic effect on aquatic organisms. By the blanketing of the substrate with settling solids, or removal of oxygen from the water, the environment is rendered unsuitable for some members of the ecosystem. In some cases, production of valuable forms may be reduced because of unsuitable habitat or lack of food organisms; in other cases, the production may be unaffected even though there is a change in population structure.

4.2.3 Biostimulation tests

Effects of added nutrients may be indirect, such as toxicant production from "red tides" or deoxygenation from algal blooms. The initial test for biostimulation is the same as that mentioned under section 4.1, Direct Response Tests, but secondary monitoring may be necessary to test for toxicity to associated biota or to human consumers.

4.3 Recommended References for Test Procedures

All of the reference works listed below contain descriptions of reliable methods. The publication with methods appropriate to a particular situation may be found by reading the titles and the added comments. Basically, the recommended test procedures are similar in all publications. A basic critical evaluation of the methods may be found, if desired, in reviews by Sprague (1969, 1970, 1971).

American Public Health Association, American Water Works Association, Water Pollution
1976 Control Federation, Standard methods for the examination of water and wastewater. 14th edition. American Public Health Association, Washington, D.C. (Available from APHA, 1015 Eighteenth Street, N.W., Washington, D.C. 20036, U.S.A. Greatly expanded coverage of bioassays compared to the 13th edition. Gives general principles and methods, and also separate detailed methods for several marine invertebrates and algae).

American Society for Testing and Materials, Standard practice for conducting acute
in press toxicity tests with fish, macroinvertebrates and amphibians. (Should be available in 1977 from the American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pennsylvania 19013, U.S.A. A short, specific outline of how to do bioassays. Basically a freshwater document but applies to marine work).

Committee on Methods for Toxicity Tests with Aquatic Organisms, Methods for acute toxicity
1975 tests with fish, macroinvertebrates and amphibians. United States Environmental Protection Agency, Ecological Research Series, EPA-660/3-75-009:61 p. (Available at no cost for single copies from Office of Public Affairs, U.S. EPA, 200 S.W. 35th St., Corvallis, Oregon 97330, U.S.A. This methodology will probably be the basis for the forthcoming ASTM procedures).

Environmental Protection Agency, Bioassay procedures for the ocean disposal permit program.
1976 United States of America, Environmental Protection Agency, Office of Research and Development, Environmental Research Laboratories - Gulf Breeze - Narragansett - Corvallis. EPA-600/9-76-010:96 p. (Available from EPA, Washington. Includes methods for testing toxicity to invertebrates).

European Inland Fisheries Advisory Commission, Report on fish toxicity testing procedures.
1974 European Inland Fisheries Advisory Commission. Working Party on Toxicity Testing Procedures. EIFAC Technical Paper No. 24, 25 p. FAO, Rome. (Gives some details of freshwater tests applicable to marine situations. Also includes excellent general guidance and was a base for preparing the present manual).

FAO, Lectures presented at the Fourth FAO/SIDA Training Course on Aquatic Pollution in
1976 Relation to Protection of Living Resources - Bioassays and Toxicity Testing, Lysekil, Sweden, 13 October - 29 November 1975. Supplement to the Report. Rome, TF-INT 173 (SWE), Suppl.1:237 p. (Available from FAO at no charge. A series of chapters by different authors. Those on details of methodology are especially recommended).

Marine Technology Society, 1974 Marine Bioassays. Workshop proceedings. Marine Technology Society, 1730 M Street, N.W., Washington, D.C. 20036, U.S.A. 308 p. (Available from the society for $18.00. Includes methods and detailed background rationale for different kinds of bioassays and organisms).

Perkins, E.J., 1972 Some methods of assessment of toxic effects upon marine invertebrates. Proc.Soc.Anal.Chem., 9:105-14. (Readable examples of difficulties with invertebrate tests in the laboratory, how to avoid them, and desirability of long-term rather than short-term tests).

4.4 Types of Procedures to be Used for Specific Purposes

A correlation can now be made between the different types of test procedures and the purposes to which they can be put. This relationship is shown in Table I, where the subdivision is initially by the type of effect, direct or indirect, and then by the nature of the response measured. The main types of test procedure for measuring each response and the major purposes for which they are used are then listed in the last two columns. Thus, screening tests can be made by the following procedures:

1. (a) single-sample bioassay: static or continuous flow, short-term, using direct lethal effects on individuals as the measured response;

6. (a) single-sample bioassays with aquatic invertebrates and vertebrates; short-term: change of behaviour, activity, respiration, growth, as the measured direct sub-lethal response;

7. (a) single-sample bioassays with algae and bacteria; short-term: effect on growth, reproduction, respiration, biostimulation as the measured direct sub-lethal response.

5. SPECIFIC USES OF THE VARIOUS TEST PROCEDURES FOR POLLUTION IDENTIFICATION, EVALUATION AND CONTROL

This section attempts to illustrate the combinations of some of the topics covered above in sections 2, 3 and 4.

There is a logical sequence of biological testing procedures required for the assessment of potential damage arising from a proposed discharge, and for subsequent monitoring and regulation. The first step is to obtain basic information on the acute toxicity of the pollutant; from the data provided by screening tests (for example the short-term LC50 and the shape of the graphical concentration/response curve) and the maximum concentration of the pollutant likely to occur in the environment, together with all other relevant chemical, physical and hydrographical data, the decision is taken on the necessity for further tests to obtain a more accurate measure of the degree of risk involved. The secondary procedures are described as tests to obtain water quality criteria, which initially will involve long-term tests on the lethal response of appropriate organisms, followed, if necessary, by a variety of relevant sub-lethal tests.

The following summary lists the basic biological procedures required for the evaluation of the potential risk arising from pollutional sources, as listed in Section 2, and for their subsequent control and regulation where necessary. This summary is meant to serve as a general guide only, and some flexibility has to be maintained in order to allow for an evaluation of discharges of unusual chemical or physical character, or specific biological effects. The numbers against each bioevaluation purpose refer to the types of procedure listed in Table I.

Table I

General description of major categories of bioevaluation procedures

Type of Effect	Response Measured	Type of Procedure		Major Purpose
Direct action on individuals or populations	Lethal effect on individuals	1	Single-sample bioassay, static or continuous-flow; short-term (a) To produce an LC50 or median survival time; (b) single-dilution, pass-fail result	 (a) Screening test* (b) Regulatory test
		2	Single-sample bioassay, static or continuous-flow; long-term	To prepare water quality criteria
		3	On-line effluent toxicity bioassay	To prepare water quality criteria
		4	On-line early-warning toxicity test	Monitoring effluent quality*
		5	In situ toxicity test	Monitoring effluent/ environment quality*
	Sub-lethal effect on individuals	6	Single-sample bioassays with vertebrates and invertebrates (a) Behaviour, activity, respiration, growth: short-term, to produce an EC50, or other measure of toxicity (b) bioaccumulation/loss studies, reproduction: long-term	 Screening tests To prepare water quality criteria*
		7	Single-sample bioassays with algae and bacteria: growth, reproduction, respiration, biostimulation studies (a) Short-term (b) long-term	 Screening test to prepare water quality criteria*

Table I
(cont'd)

Type of Effect	Response Measured	Type of Procedure		Major Purpose
		8	On-line, or in situ, effluent toxicity test, respiration, activity	To prepare water quality criteria
		9	On-line early-warning, or in situ, toxicity test: respiration, activity	Monitoring effluent/ environment quality*
	Lethal or sub-lethal effect on cells, tissues or organs	10	Single-sample bioassays: biochemical, physiological, histopathological effects	To prepare water quality criteria
		11	On-line early-warning toxicity test	Monitoring effluent/ environment quality*
	Changes in population structure	12	Ecological surveys	To assess effects of pollutants on eco-systems (monitor/ surveillance)
Indirect action on individuals or populations	Changes in population structure	13	Ecological surveys	To assess effects of pollutants on eco-systems (monitor/ surveillance
	Effects on predators (including man)	14	Bioaccumulation tests	Protection of higher trophic levels
		15	Organoleptic tests	Acceptance for human food

*Can also be modified for regulatory purposes

5.1 Fixed-point Discharge

Continuous discharge

Before discharge is made or to investigate existing discharge:

(i) Screening test (1a, 6a, 7a);

(ii) if appropriate[1/], tests to prepare water quality criteria (2, 3, 6b, 7b, 8 and 10);

(iii) indirect effects (14 and 15).

During period of discharge:

(i) Monitor effluent quality (4, 5, 9 and 11);

(ii) ecological surveys (12, 13);

(iii) regulatory tests (1b or modified tests, 1a, 4, 5, 6b, 7a, 9 and 11).

Intermittent discharge

Before discharge is made, or to investigate existing discharge:

(i) Screening tests (1a, 6a, 7a);

(ii) if necessary, tests to prepare water quality criteria (2, 6b, 7b and 10, as appropriate);

(iii) indirect effects (14 and 15).

During period of discharge:

(i) Monitor effluent/waste quality (4, 5, 9 and 11 as appropriate);

(ii) ecological surveys (12 and 13);

(iii) regulatory tests (1b or modified tests, 1a, 4, 5, 6a, 7, 9 and 11, as appropriate).

5.2 Variable Location/Random-point Discharge

Continuous discharge

Before discharge is made, or to investigate existing discharge:

(i) Screening tests (1a, 6a, 7a);

(ii) if necessary, tests to prepare water quality criteria (2, 6b, 7b and 10, as appropriate);

(iii) indirect effects (14 and 15).

During period of discharge:

(i) Monitor effluent/waste quality (4, 5, 9 and 11, as appropriate);

(ii) ecological surveys (12 and 13).

1/ If the data from screening tests indicate that the approximate margin of safety between discharge concentration and test organism sensitivity is likely to be small, further tests are required to identify the margin of safety with greater accuracy using appropriate test species. The smaller the margin of safety the more complex and detailed are the test procedures necessary.

Intermittent discharge

Before discharge is made, or to investigate existing discharge:

(i) Screening tests (1a, 6a, 7a);

(ii) if necessary, tests to prepare water quality criteria (2, 6b, 7b and 10, as appropriate);

(iii) indirect effects (14 and 15).

After discharges have been made:

(i) Ecological surveys (12 and 13).

5.3 Diffuse Pollution

(i) Ecological surveys (12 and 13);

(ii) identify pollutants, using tests 1a, 2, 6, 7a and 10, as appropriate.

Detailed examples of the above approaches for the evaluation of the biological effects of pollution arising from types 1, 3 and 5 are given in Appendix 1.

6. SELECTION OF TEST ORGANISMS

To achieve maximum information from the different types of bioevaluation, it is necessary to select the most appropriate test organisms. It is important to consider that different periods of laboratory acclimation are required, depending on the species, and it is most important that the test organisms be carefully handled, undamaged, healthy and of uniform age or size. Selection of the appropriate test organisms should be based on the following criteria.

6.1 General Considerations

Availability: The organisms to be tested should be readily available in the region, in sufficient numbers and easily collected without damage. Problems of transportation should be considered when selecting species for testing.

Maintenance: The animals must be capable of being kept in a healthy condition for a long period of time, the length of time varying with the species and its sensitivity. Organisms should be kept in conditions as natural as possible as to dissolved oxygen concentration, temperature, salinity, etc. Sufficient space must be provided to avoid stress due to crowding and cannibalism. Actively swimming species, such as fish and crustaceans require more space than other species.

Size: In general, test organisms should be small enough to be kept in the test apparatus in sufficient numbers to permit good statistical treatment of data.

Biology of test organism: a good knowledge of the biology of the test organism is essential for a proper interpretation of test results and should be acquired from the literature or from observations prior to initiation of tests.

Salinity tolerance: Euryhaline species are often useful since they can be tested over a broad range of salinity regimes without added stress.

6.2 Organisms for Screening and Regulatory Tests

Non-standard screening test organisms: Appropriate species are those which may be collected locally as wild specimens or as specimens which have been cultured in the laboratory or in the field. These organisms are particularly useful in establishing the

relative toxicity of two or more pollutants and for screening effluents containing mixed or unknown compounds. If an extensive testing programme is contemplated, laboratory populations of local species of fish and invertebrates should be maintained as in-house "standard" strains so that the most sensitive life-stages will be available as required for bioevaluation.

Standard screening test organisms: The ideal characteristic of a standard test organism is that it has been cultured for more than one generation and its biological responses have been reasonably well established. Standardized test organisms are especially useful for comparisons of results and intercalibration exercises between laboratories. A few strains of suitable test species have been maintained long enough to establish their genetic backgrounds, and research is in progress to establish standard cultures of crustaceans and fish.

Regulatory test organisms: Where possible, regulatory tests should be made with species which can be obtained locally and which are available at a uniform size and constant sensitivity throughout the year.

6.3 Organisms for Other Bioassays

Selection of the most appropriate test organisms is often dependent upon a known or anticipated pollution source and the populations of local species in the vicinity of the disposal site. The selection should be based on the following criteria.

Type of organism: Representatives of different groups, such as algae, annelids, crustaceans, molluscs and fish should be considered for test organisms because of the wide range of their susceptibility to different pollutants.

Behaviour: Representatives of different types of behaviour, such as locomotion, feeding, shell or tube formation, closure of shells, withdrawal activity or byssus thread formation should be considered in the selection of test organisms because they are useful as rapid indicators of stress or sub-lethal effects.

Trophic level: Representatives of the different trophic levels, such as primary producers, herbivores, detrital feeders and carnivores, should be considered in the selection of organisms, especially for bioaccumulation tests.

Commercially important species: Representatives of the commercially important species of the area, such as macrophytes, crustaceans, molluscans and fish should be considered in the selection of test organisms because of their economic value.

Sensitive stage: The younger stages in the life cycle of the organisms - the egg, larva and the juvenile - are often more sensitive than the adults to toxicants. It is important that these younger stages of commercially important species be used for bioassays. In general, the most sensitive life stage of these organisms is in the period just after the yolk has been consumed or that following the molting period in crustaceans.

Growth rates: An increase in body weight is an indication of a healthy organism. The test organism must be capable of being fed a standard diet at a regular rate or, in the case of the filter feeders, be supplied with unfiltered, flowing sea water.

Life cycle: Tests for the ability to complete a life cycle provide good indications of possible sub-lethal effects. It is desirable to select those organisms with a short life cycle. The relation between the number of eggs produced and concentration of toxicant can be an indicator of impairment of reproductive success.

6.4 Special Characteristics of Some Test Groups

Most groups of test organisms have certain characteristics that make them more or less desirable for bioassays. Algae and zooplankton, for example, are convenient because of their small size, and it is possible to conduct many tests with them in a small space. They have a very short life cycle and their nutritional requirements have been established. They are good for bioaccumulation studies, since they are on the lowest level of the trophic pyramid. On the other hand, their smallness makes microscopes and other specialized equipment necessary for their identification and counting. Annelids such as polychaetes are useful for many of the same reasons and have the advantage of larger size.

Echinoderms are useful because of their wide distribution in littoral areas and the simplicity of obtaining large numbers of eggs and larvae from them. Crustaceans, such as amphipods and decapods, are useful because of their sensitivity to synthetic organic compounds; they have increased sensitivity during the molting stage. Many species are cannibalistic, so they must be isolated during tests.

Molluscs have many advantages as test animals. They are easy to handle for growth measurements, they are good bioaccumulators and usually they are easy to collect. They can be maintained in the laboratory so as to produce larvae when required for tests throughout the year. Their chief disadvantage is that they require a good supply of unfiltered seawater. The adults of many species cannot be used for lethal toxicity tests since the condition of the animal inside the shell cannot be observed.

Fish are a major test group because of their economic value. They have many practical advantages over other test organisms. Their biology is well known and their responses to pollution have been thoroughly documented. In general, fish are sensitive to most types of pollution but they have the disadvantage of being easily stressed. Special care must be used in handling them from the time of capture, in transporting them to the laboratory and in providing adequate holding facilities and diets until they are used for tests.

It is important to note that many animals have a delayed response to pollutants and they should be maintained for several days after a test to observe this possible response.

It must be emphasized that satisfactory bioassays can be done only with healthy, relatively unstressed test animals.

6.5 Selected Bibliography

The choice of specific organisms for toxicity tests is dependent on local conditions. The following references indicate a few of the organisms that have been used satisfactorily in both acute and chronic toxicity testing, and in many cases species are listed.

Algae

Antia, N.J. and J.Y. Cheng, 1975 Culture studies on the effects from borate pollution on the growth of marine phytoplankters. J.Fish.Res.Board Canada, 32:2487-94

North, W.J., G.C. Stevens and B.B. North, 1972 Marine algae and their relation to pollution problems. In Marine pollution and sea life, edited by M. Ruivo. West Byfleet, Surrey, Fishing News (Books) Ltd., pp. 330-40

Ukeles, R., 1962 Growth of pure cultures of marine phytoplankton in the presence of toxicants. Appl.Microbiol., 10(6):532-7

Bacteria

Bonde, G.J., 1966 Bacteriological methods for the estimation of water pollution. Health Lab. Sci., 3(2):124-8

Trudgill, P.W., R. Widdus and J.S. Rees, 1971 Effects of organochlorine insecticides on bacterial growth respiration and viability. J.Gen.Microbiol., 69:1-13

Annelida

Reish, D.J., 1970 The effects of varying concentrations of nutrients, chlorinity and dissolved oxygen on polychaetous annelids. Water Res., 4:721-35

_____, 1972 The use of marine invetebrates as indicators of varying degrees of marine pollution. In Marine pollution and sea life, edited by M. Ruivo. West Byfleet, Surrey, Fishing News (Books) Ltd., pp. 203-7

Crustacea

Bahner, L.H., C.D. Craft and D.R. Nimmo, 1975 A saltwater flow-through bioassay method with controlled temperature and salinity. Progr.Fish Culturist, 37(3):126-9

Molluscs

Lowe, J.I., P.D. Wilson, A.J. Rick and A.J. Wilson, Jr., 1970 Chronic exposure of oysters to DDT, toxaphene and parathion. Proc.Nat.Shellf.Assoc., 61:71-9

Fish

Schimmel, S.C. and D.J. Hansen, 1975 Sheepshead minnow (Cyprinodon variegatus): An estuarine fish suitable for chronic (entire life-cycle) bioassays. In Proceedings of the Twenty-eighth Annual Conference of Southeastern Association of Game and Fish Commissioners, November 17-20, 1974, White Sulphur Springs, Virginia. pp. 292-8

General

Hansen, D.J., 1974 Arochlor^{R} 1254: Effects on composition of developing estuarine animal communities in the laboratory. Contribut.Mar.Sci., 18:19-33

Nimmo, D.R., D.J. Hansen, J.A. Couch, N.R. Cooley, P.R. Parrish and J.I. Lowe, 1975 Toxicity of Arochlor^{R} 1254 and its physiological activity in several estuarine organisms. Arch.Environ.Contam.Toxicol., 3(1):22-39

7. SALINITY OF TEST SOLUTIONS

An especially important part of interpreting marine toxicity tests is consideration of the salinity of dilution waters and of the effluent itself. The toxicity bioassay of a sewage or industrial effluent involves testing different concentrations, obtained by dilution with a suitable water, usually the receiving water or a similar one. The salinity of the dilutions may markedly influence the toxicity of pollutants present.

If the effluent has a salinity similar to the receiving water, the latter can be used for dilution and no problems exist as the salinity of the various dilutions will be uniform.

If the effluent is essentially freshwater, and no great dilution is required, the use of saltwater as a diluent may cause exceedingly variable salinities in the solutions of varying concentration, and may sometimes be too low for typical marine organisms. Euryhaline test organisms, tolerant of high and low salinities might then be used with advantage. Even so, the result of a test of limited duration (e.g. an acute toxicity bioassay) performed with such organisms without adjustment of the salinity may not be a satisfactory basis for estimating the safe concentration of the effluent in saline receiving water. The difficulty can be overcome by addition of sea salts to the freshwater effluent until its salinity equals that of the saltwater used as a diluent. Any suitable marine organism then can be used as the test organism and the tests performed at a constant, high salinity. If sea salts are obtained from a commercial distributor, the investigator must make sure that no chelating agents have been used in the formulation. Careful testing of the artificial seawater's suitability for the organism is desirable, since some formulations may be poor for culturing marine organisms, especially invertebrates.

In some situations involving organisms which are exposed alternately to salt and freshwater, a series of tests at different salinities might be desirable. Osmotic stress at a particular point in an estuary can add to pollutant stress.

For monitoring tests of non-saline effluents, fresh dilution waters and freshwater test organisms sometimes are used even though the receiving waters are saline. The appropriateness of such tests should be checked by performing experiments with marine organisms at salinities characteristic of the receiving water, using sea salts to produce a saline dilution water.

8. INTERPRETATION OF RESULTS

This section describes some general difficulties in interpreting bioassay results common to many of the tests listed above.

8.1 General Aspects

Water pollution can impair the production of aquatic organisms valued by man or render them unsuitable for use as food; it can also increase the production of organisms so that they become excessively abundant and interfere with beneficial uses of water. The need to prevent these undesirable effects is the practical reason for undertaking bioevaluation of water pollutants.

8.2 Ecological Significance of Test Results

Estimates of harmless concentrations of pollutants in the field have been based mostly on results of laboratory experiments. It should be understood that such experiments, performed under entirely unnatural conditions, cannot be relied upon to reveal exactly how organisms or their production will be affected by the introduction of the tested pollutants into the natural environment. The effects of poisons on the growth of animals in aquaria where food is supplied in abundance and consumed with little or no effort can be very different from effects in nature, where the food supply is limited and the animals must expend much energy in seeking and capturing their prey. Similarly, sub-lethal concentrations may render a species more susceptible to a predator. Avoidance reactions of animals to water quality differences under highly artificial conditions in the laboratory may not occur in more natural situations where concentration gradients are much more gradual than those encountered by animals in the laboratory tests. Territorial or feeding behaviour may also override avoidance reactions. Because close simulation of natural conditions in the laboratory is not usually feasible, conclusions drawn from the laboratory experiments should be verified through pertinent observations made in the field whenever such verification is possible.

Beyond this, it must be stressed that the production of any species valued by man is dependent on the functioning of the entire ecosystem. With bioassays, it is not feasible to test sub-lethal effects on every organism in the community. The approach is usually to test with a representative organism, or a series of sensitive organisms such as those recommended in section 6. It is then assumed that protection of the selected test organisms will also protect the community of organisms. One may not assume, however, that injury to any one of the many species that contribute to the nutrition of organisms valued by man will necessarily have an adverse effect on the production of the valuable species. An increase in production of tolerant forms in waters receiving wastes sometimes fully compensates for an impairment of the production of more susceptible food organisms. Therefore, observations and experiments on entire communities of marine organisms may be necessary to demonstrate adverse effects on valuable species.

Concentrations of pollutants that are lethal to marine organisms are obviously incompatible with their unimpaired production, but production can be greatly reduced or entirely prevented by concentrations that are tolerated indefinitely by the fully developed organisms. These sub-lethal concentrations can interfere with reproduction by reducing the fecundity of adults or by causing developmental abnormalities and death of sensitive embryos or larvae. They can also interfere with growth of the young by reducing feeding activity, limiting the quantity of food that can be consumed and assimilated, or impairing the efficiency of metabolic utilization of the assimilated food and its conversion into new body tissues. Also, fish sometimes may be repelled by otherwise harmless concentrations of pollutants and may consequently avoid areas otherwise suitable for them. Migratory fauna thus can be denied necessary access to nursery areas and spawning grounds.

If such effects on reproduction are widespread, they can be of major importance. However, some reduction in reproductive capacity, especially if it is local, may not be of overall significance. Reproduction may not be a factor limiting production in a system, or immigration from unaffected areas may compensate for the reduction.

One should not assume that any detectable and measurable response of organisms to a pollutant is ecologically significant. Pollutional conditions that can be reasonably supposed to cause premature death, increased susceptibility to predation or marked reduction of reproductive capacity, activity or growth of valuable marine organisms in their natural environment are obviously undesirable. On the other hand, some responses, such as changes of enzymes or blood parameters, may or may not be ecologically significant; they can be merely adaptive and not indicative of any real injury to the organisms affecting their production. Only when a definite correlation has been demonstrated between the magnitude of physiological change and the degree of ecological impairment, can practical importance or predictive value be attributed to the measurement of such responses.

Axenic cultures of algae have shown a remarkable degree of adaptation in bioassays with some pollutants, given sufficient time. Hence, results of short-term algal bioassays may be misleading in terms of eventual primary production (Stockner et al., 1975). In such cases, the purpose of the test would decide the duration.

8.3 Measurement of Toxicity

The concentration of a toxic pollutant just sufficient to kill a percentage (e.g. 50%) of organisms of a given species in the natural environment within a given exposure period usually can be estimated reliably enough through properly performed laboratory tests. The highest concentration that does not prove lethal to any individual in a tested sample of a population of organisms is not very meaningful, for it is not a reliable estimate of the corresponding value for the entire population. Unlike the LC50, this concentration, sometimes reported as the "LC0" tends to decrease with increase in size of the sample, for it depends on the resistance of the most susceptible individual

organism that happens to be included in the sample. A value designated "LC100" obviously is not any more meaningful but estimates of LC10 and LC90 can be instructive and useful. Since tolerance limits such as the LC50 vary with the duration of exposure, exposure periods must be specified in reporting such data (e.g. 96 h LC50). However, if the LC50 is found or can be reasonably assumed to remain virtually constant with increase of exposure time beyond some sufficiently long exposure period (which may be only a day or two or very much longer), the constant (minimal) value can be reported as the threshold LC50. Though highly desirable, the determination of lethal thresholds is not always feasible because of excessive length of the tests sometimes required.

In acute toxicity bioassays, fish and crustacea may be incapacitated, perhaps lying on the bottom of the tank for some time before mortality occurs. Such animals may be in effect "ecologically dead", and use of turnover time as an additional experimental endpoint may be meaningful. In many toxicity tests, the responses recorded are sub-lethal effects, such as changes in growth, loss of equilibrium, partial paralysis or complete immobilization. In such cases, it is appropriate to speak of the Median Effective Concentration, EC50, but the effect and its definition must always be reported.

8.4 Application Factors

Maximal concentrations of poisons that can be tolerated by aquatic organisms for limited periods of time are usually many times greater than those that are entirely harmless to them. The ratio of the maximum apparently harmless concentration of a poison to the concentration that is lethal, after a given exposure period, to 50 percent of test animals (median lethal concentration, LC50, or median tolerance limit, TL50) has been termed the "application factor". It has been proposed that, in the absence of specific information on sub-lethal effects, the concentration of a toxicant that is safe enough for a particular species in a given water be estimated by multiplying the LC50 determined for that species in the same water by the appropriate application factor. Recommended application factors pertaining to various toxic pollutants have been published (National Academy of Sciences - National Academy of Engineering, 1973) but many of these are based on very limited experimental data and need verification. Most of these values fall within the range of 0.01 to 0.10. That is, the "safe" level may be estimated as 0.01 to 0.10 of the LC50. Sometimes an application factor of 0.001 may be appropriate. The choice of application factors for regulatory purposes should not be entirely independent of socio-economic considerations, such as the value of a fishery to be protected, which determines how much damage or risk of damage to that fishery is to be considered acceptable.

8.5 Concentration Versus Amount of Toxicants

Even if two effluents have the same level of toxicity (i.e. LC50), it is obvious that the relative volumes discharged are of profound importance in assessing potential damage to the environment which receives them. A formalized method of evaluating the "toxicity emission rate" has come into use recently (California Water Resources Control Board, 1972). An explanation of the method and its use is given in Appendix 2.

9. REFERENCES

ACMRR Working Party on Biological Accumulators, Report of the first session, FAO Head-
1975 quarters, Rome, Italy, 9-13 December 1974. FAO Fish.Rep., (160):18 p.

_____, Report of the second session, FAO Headquarters, Rome, Italy, 21-25 July
1975a 1975. FAO Fish.Rep., (165):9 p.

ACMRR/IABO Working Party on Ecological Indices of Stress to Fishery Resources, Indices
1976 for measuring responses of aquatic ecological systems to various human influences. FAO Fish.Tech.Rep., (151):66 p.

American Public Health Association, American Water Works Association, Water Pollution
1976 Control Federation, Standard methods for the examination of water and wastewater. 14th edition. Washington, D.C., American Public Health Association

American Society for Testing and Materials, Standard practice for conducting acute
in press toxicity tests with fish, macroinvertebrates and amphibians. Philadelphia, American Society for Testing and Materials

Antia, N.J. and J.Y. Cheng, Culture studies on the effects from borate pollution on the
1975 growth of marine phytoplankters. J.Fish.Res.Board Can., 32:2487-94

Bahner, L.H., C.D. Craft and D.R. Nimmo, A saltwater flow-through bioassay method with
1975 controlled temperature and salinity. Progr.Fish Culturist, 37(3):126-9

Bonde, G.J., Bacteriological methods for the estimation of water pollution. Health Lab.
1966 Sci., 3(2):124-8

California Water Resources Control Board, Water quality control plan for ocean waters of
1972 California. State of California, Water Resources Control Board, 18 p.

Committee on Methods for Toxicity Tests with Aquatic Organisms, Methods for acute
1975 toxicity tests with fish, macroinvertebrates and amphibians. United States Environmental Protection Agency, Ecological Research Series, EPA-660/3-75-009: 61 p.

Doudoroff, P. and D.L. Shumway, Dissolved oxygen requirements of freshwater fishes.
1970 FAO Fish.Tech.Pap., (86):291 p.

Environmental Protection Agency, Bioassay procedures for the ocean disposal permit program.
1976 Washington, D.C., United States of America, Environmental Protection Agency, Office of Research and Environment, Env.Res.Labs - Gulf Breeze - Narragansett - Corvallis. EPA-600/9-76-010:96 p.

European Inland Fisheries Advisory Commission, Report on fish toxicity testing procedures.
1974 EIFAC Working Party on Toxicity Testing Procedures. EIFAC Tech.Pap., (24): 25 p.

FAO, Lectures presented at the Fourth FAO/SIDA Training Course on Aquatic Pollution in
1976 Relation to Protection of Living Resources - Bioassays and Toxicity Testing, Lysekil, Sweden, 13 October - 29 November 1975. Supplement to the Report. Rome, TF-INT 173 (SWE), Suppl.1:237 p.

Geckler, J.R., Validity of laboratory tests for predicting copper toxicity in streams.
in press EPA Res.Rep.Ser.

Hansen, D.J., Arochlor^{R} 1254: Effect on composition of developing estuarine animal
1974 communities in the laboratory. Contrib.Mar.Sci., 18:19-33

IMCO/FAO/Unesco/WMO/WHO/IAEA/UN Joint Group of Experts on the Scientific Aspects of Marine
1975 Pollution (GESAMP), Report of the seventh session. Rep.Stud.GESAMP, (1): pag.var.

Lowe, J.I. et al., Chronic exposure of oysters to DDT, toxaphene and parathion. Proc.
1970 Nat.Shellf.Assoc., 61:71-9

Marine Technology Society, Marine bioassays. Workshop proceedings. Washington, D.C., 1974 Marine Technology Society, 308 p.

National Academy of Sciences-National Academy of Engineering, Water quality criteria, 1973 1972. U.S. National Academy of Sciences-National Academy of Engineering. U.S. Environmental Protection Agency, Washington. Ecol.Res.Ser., EPA-R3-73-033, 594 p.

Nimmo, D.R. et al., Toxicity of Arochlor^{R} and its physiological activity in several 1975 estuarine organisms. Arch.Environ.Contam.Toxicol., 3(1):22-39

North, W.J., G.C. Stevens and B.B. North, Marine algae and their relation to pollution 1972 problems. In Marine pollution and sea life, edited by M. Ruivo. West Byfleet, Surrey, Fishing News (Books) Ltd., pp. 330-40

Perkins, E.J., Some methods of assessment of toxic effects upon marine invertebrates. 1972 Proc.Soc.Anal.Chem., 9:105-14

Portmann, J.E. (Ed.), Manual of methods in aquatic environment research. Part 2. 1976 Guidelines for the use of biological accumulators in marine pollution monitoring. FAO Fish.Tech.Pap., (150):76 p.

Reish, D.J., The effects of varying concentrations of nutrients, chlorinity and dissolved 1970 oxygen on polychaetous annelids. Water Res., 4:721-35

_____, The use of marine invertebrates as indicators of varying degrees of marine 1972 pollution. In Marine pollution and sea life, edited by M. Ruivo. West Byfleet, Surrey, Fishing News (Books) Ltd., pp. 203-7

Schimmel, S.C. and D.J. Hansen, Sheepshead minnow (Cyprinodon variegatus): An estuarine 1975 fish suitable for chronic (entire life-cycle) bioassays. In Proceedings of the Twenty-eighth Annual Conference of Southeastern Association of Game and Fish Commissioners, 17-20 November 1974, White Sulphur Springs, Virginia, pp. 392-8

Sprague, J.B., Measurement of pollutant toxicity to fish. 1. Bioassay methods for 1969 acute toxicity. Water Res., 3:793-821

_____, Measurement of pollutant toxicity to fish. 2. Utilizing and applying bio- 1970 assay results. Water Res., 4:3-32

_____, Measurement of pollutant toxicity to fish. 3. Sublethal effects and "safe" 1971 concentrations. Water Res., 5:245-66

Sprague, J.B. and D.E. Drury, Avoidance reactions of salmonid fish to representative 1969 pollutants. Advances in Water Pollution Research, Proceedings of the Fourth International Conference, Oxford, Pergamon Press, Vol. 1, p. 169-79

Stockner, J.G., D.D. Cliff and K. Munro, The effects of pulpmill effluent on phytoplankton 1975 production in coastal waters of British Columbia. Can.Fish.Mar.Serv., Tech.Rep., (578):99 p.

Trudgill, P.W., R. Widdus and J.S. Rees, Effects of organochlorine insecticides on 1971 bacterial growth, respiration and viability. J.Gen.Microbiol., 69:1-13

Ukeles, R., Growth of pure cultures of marine phytoplankton in the presence of toxicants. 1962 Appl.Microbiol., 10(6):532-7

Warren, C.E., Biology and water pollution control. Philadelphia, W.B. Saunders Co., 1971 434 p.

Woelke, C.E., Measurement of water quality with the Pacific oyster embryo bioassay. 1967 ASTM Tech.Publ., (416):112-20

Appendix 1

EXAMPLES OF INVESTIGATIONAL PROGRAMMES APPROPRIATE TO THREE TYPES OF POLLUTION SOURCES

This Appendix contains examples of an idealized approach to the type of investigation required for three types of pollution sources as outlined in section 2.

A. Fixed-point discharge: continuous

B. Variable location/random-point discharge: intermittent

C. Diffuse pollution.

These examples are, of necessity, only a broad guide to the methodology which may be used, since, for any specific situation, a considerable amount of flexibility of approach is required as the investigation proceeds, with each progressive stage being highly dependent on the results already obtained.

Example A: FIXED-POINT DISCHARGE: CONTINUOUS

Situation

Investigation for regulatory purposes is needed of an existing, fixed-point discharge of a possibly toxic (but not oxygen-depleting) industrial effluent into an estuary.

Investigation Programme (one possible approach)

1. The Mixing Zone and Protection Level

Define a reasonable mixing (dispersion or dilution) zone for the effluent and decide what uses of the receiving water outside this zone must be protected and what is an appropriate "level of protection" for each use. This must be done with careful attention to the commercial and recreational value of local populations of aquatic animals and to other pertinent social and economic considerations. The mixing zone is that area within which some ecological or other damage that is deemed unacceptable elsewhere is to be tolerated. The concept of "levels of protection" of different water uses such as fisheries has been presented and discussed in several recent publications (Doudoroff and Shumway, 1970, p. 255-68; Warren, 1971, p. 15-23; National Academy of Sciences-National Academy of Engineering, 1973, p. 131-4).

By using a dye or other suitable "tracer" or the results of salinity measurements, determine the concentration of the effluent (fraction by volume) not likely to be often materially exceeded outside the boundary of the defined, acceptable mixing zone.

2. Bioassay Programme and Ecological Surveys

By consulting literature, learn what is known about wastes of the same general kind, their chemical composition, toxicological properties, etc. Verify the pertinence of this information through chemical analysis of the waste, if possible. Perform single-sample, acute toxicity bioassays of the effluent (Table I; 1a) with appropriate test animals to estimate the relevant LC50 values if the effluent has measurable acute toxicity.

On the basis of all the above considerations and other pertinent information, decide what fraction of an LC50 can be reasonably deemed acceptable at the boundary of the defined mixing zone, i.e. what application factor is probably appropriate. For example, 0.1 may be taken as a reasonable tentative application factor for the 96 h LC50, having been successfully used as a regulatory standard for various wastewater discharges to some estuaries from submerged outfalls. For persistent, slowly acting toxicants, however, an application factor of 0.01 may be more appropriate if a high level of protection of aquatic life is desired.

If the concentration of the effluent outside the defined mixing zone is found to be always well below the product of the determined LC50 and the application factor (a.f.) deemed appropriate (LC50 x a.f.), and if the effluent is believed not to contain any substances whose harmful effects in receiving waters can be expected to have little or no relation to the acute toxicity of the effluent, no further bioassays need to be undertaken. However, chemical tests of the receiving water outside the mixing zone and an ecological survey (Table I; 12, 13) designed to verify the conclusion that the waste discharge is harmless are advisable.

If, on the other hand, the effluent concentrations at the boundary of the mixing zone are found to approach or exceed the product of the LC50 and the chosen application factor or if potentially harmful substances are known to be present for whose detection and measurement acute toxicity bioassays are unsuitable, then appropriate additional bioassays (Table I; 2, 3, 6, 7, 8, 10) and chemical and ecological surveys (12, 13) of the receiving water are essential. The nature of these investigations, directed toward the preparation of water quality criteria, will depend on the nature of the dangerous pollutants found to be present. For example, organoleptic tests (Table I; 15) may be undertaken if serious tainting of the flesh of fish caught in the vicinity of the outfall has been noted. If the undiluted effluent does not prove acutely toxic but its concentration at the boundary of the defined mixing zone is much more than 10% by volume, bioassays of longer duration and more sensitive than the acute toxicity tests may be needed to show that serious sublethal, toxic effects on aquatic life outside the mixing zone are unlikely, unless there is sufficient evidence from field (ecological) studies. If the undiluted effluent is found to be very rapidly fatal to fish, its dilution in the mixing zone to a harmless level may not be sufficient to prevent damage to fish life. The possibility that fish entering the mixing zone are being killed then should be considered and investigated through appropriate observations in the field or "repellence bioassays", which may even reveal attraction of fish by the toxic effluent.

Example B: VARIABLE LOCATION/RANDOM-POINT DISCHARGE: INTERMITTENT

Situation

An industry has applied for a permit to dispose of a large amount of waste at sea where the water is not polluted.

Investigation Programme

First obtain a large enough sample of the material for testing purposes, and determine the chemical composition of the waste as much as possible. Undertake a thorough search of the literature to determine if other studies have been made of similar waste disposal problems.

1. Bioassay Programme

Perform tests to determine short-term LC50 levels (Table I; 1a), using locally available animals. If the waste contains fat-soluble persistent components, it is desirable to conduct long-term tests with a flow-through system to obtain water quality criteria and

bioaccumulation data (see Table I; 2, 6, 7, and 10 as appropriate, and 14).

If the mixture has insoluble components and settleable solids, make direct observations on their effects on fish and gill breathing invertebrates.

2. Ecological Surveys

If bottom deposits are anticipated do an ecological survey of the benthic community to determine density and types of organisms before the dumping (Table I; 12).

If there are to be repeated discharges of the waste, monitor the initial dumping to observe possible immediate effects on fish populations and to determine that there has been adequate dispersion and mixing with no persistent changes in turbidity or water colour. Make ecological surveys (Table I; 12) 7-10 days after the waste is dumped to detect possible changes in the benthic community. Under some conditions, the amount of waste may be so small, or water depth so great that little damage can be expected to the benthic community, and ecological surveys would be unwarranted.

Example C: DIFFUSE POLLUTION

Situation

Industrial and domestic sewage discharges to a river give rise to coastal pollution which is affecting the breeding grounds of a commercially important fish species. The result is a decrease in fish catch.

Investigation Programme

1. Ecological Survey

To identify possible environmental effects, undertake ecological surveys (Table I; 12, 13) to identify the communities of epibenthic and benthic organisms present. Such surveys should ideally be continued for several years to establish the annual variations in composition and population densities. It is advisable also to study a reference area which is not polluted. Such a survey may indicate the location of major pollution discharges.

2. Chemical Survey

Obtain information about the volume of discharges and the concentration of pollutants which they contain from the appropriate industries and authorities, where possible. Make chemical analyses of the coastal waters to determine the concentrations of the major known pollutants. The analyses should be made at regular intervals throughout the year.

3. Bioassay Programme

Make a literature survey to obtain data on the toxicity of the known pollutants to the types of organisms found in the polluted area.

Perform screening tests (Table I; 1a) to measure the acute toxicity of the known pollutants to selected test organisms (which should be local species closely related to other well-studied organisms); if possible, fish of commercial value should be used. The toxicity of the effluents discharged should be measured to determine whether their toxicity is greater than that predicted from their chemical analysis, in order to detect the presence of unknown pollutants or synergistic interactions.

Conduct long-term tests (Table I; 2) of the major pollutants to measure the chronic toxicity of those pollutants producing effects only after long exposure.

Study sub-lethal effects of the important pollutants on behaviour, activity, respiration, reproduction, growth, bioaccumulation, etc. (Table I; 6) using the fish species affected and some other representative local species to establish water quality criteria. These tests should include studies of effects of the important pollutants on biochemical and physiological functions of the fish (Table I; 10). Of particular importance are the studies of the effects on reproduction, eggs and larval development of the affected fish species concerned, to establish water quality criteria for the breeding areas and nursery grounds.

This programme should provide data on water quality criteria for the important pollutants with particular reference to the affected species of fish.

REFERENCES

Doudoroff, P. and D.L. Shumway, Dissolved oxygen requirements of freshwater fishes. FAO
1970 Fish.Tech.Pap., (86):291 p.

National Academy of Sciences-National Academy of Engineering, Water quality criteria, 1972.
1973 U.S. National Academy of Sciences-National Academy of Engineering. U.S. Environmental Protection Agency, Washington. Ecol.Res.Ser., EPA-R3-73-033, 594 p.

Warren, C.E., Biology and water pollution control. Philadelphia, W.B. Saunders Co., 434 p.
1971

Appendix 2

CALCULATION AND USE OF THE TOXICITY EMISSION RATE

Requirements or standards regulating the discharge of wastes may or may not be related to the assimilative capacities of the receiving waters, or the amounts of dilution of the wastes after discharge. In either case, restrictions should be placed on amounts of toxic substances discharged per unit of time, and not only their concentrations in effluents. The daily discharge of a small volume of a highly toxic effluent can be less harmful to aquatic life than the discharge into the same body of a much larger volume of a less toxic effluent. A way to evaluate and compare amounts of toxic pollution contributed by different discharges has been developed and used in California (California Water Resources Control Board, 1972) and is gaining favour elsewhere.

The "toxicity concentration" in an effluent, expressed in "toxicity units" (t.u.) is taken to be the reciprocal of the median lethal concentration (LC50) expressed as a decimal fraction by volume. For example, if the LC50 of an effluent for an approved test animal is 0.25 (25% effluent) by volume, the effluents "toxicity concentration" = 1/0.25 = 4 t.u., or four times the LC50. The best procedure is to determine and use in each such computation the threshold LC50, but the 96 h LC50 has been commonly used instead.

The "toxicity emission rate" (TER) for each waste outfall is taken to be the effluents' toxicity concentration in t.u. multiplied by its flow rate (volume discharged per unit of time), expressed in any suitable discharge rate units. For example, if the flow rate of the effluent with an LC50 value of 0.25 (25%) by volume, i.e. a toxicity concentration of 4 t.u., happens to be 3 cubic metres per minute (m^3/min):

$$\text{TER} = 4 \times 3 = 12 \ \frac{\text{t.u.} \times m^3}{\text{min}}$$

The TER can also be calculated as follows:

$$\frac{\text{flow rate}}{\text{LC50}} = \frac{3}{0.25} = 12 \ \frac{\text{t.u.} \times m^3}{\text{min}}$$

The computation of TER values facilitates comparison of the environmental impacts of large and small discharges of wastewaters having widely different toxicities. As an example, five hypothetical sources of pollution will be considered:

Type of Waste	LC50		Toxicity Units	Effluent Discharge m^3/min	TER
	%	Decimal			
(1) Anti-fouling treatment of piles	0.2	0.002	500	0.001	0.5
(2) Chemical plant	1	0.01	100	0.80	80
(3) Factory	10	0.10	10	2.0	20
(4) Sewage, city of 500 000	70	0.7	1.43	140	200
(5) Petrochemical plant	90	0.9	1.11	27	30
				Total TER:	330.5

It can be seen that an arrangement of the five wastes in order of decreasing TER would be quite different from their present arrangement in order of decreasing toxicity. Number 4 in order of toxicity is first in order of TER, and number 1 in order of toxicity is fifth (last) in order of TER. Such an assessment can be especially helpful when comparisons are made of different industrial plants whose products and wastes are similar (e.g. different petrochemical plants, or different pulp and paper mills using essentially the same process but different equipment, amounts of water, etc.). Toxic pollution loads per ton of product then can be readily compared and equitably regulated. When dealing with occasional discharges (not continuous outflows), such as the dumping of barge-loads of toxic wastes, "toxicity emissions" (TE), expressed in t.u. x m^3 or other similar terms, can be computed for comparative and regulatory purposes.

If all of the five effluents listed in the foregoing table are discharged into one estuary and there are no other sources of toxic pollution, the rate of total toxicity emission to the estuary (total TER) can be seen to be about

$$330 \ \frac{\text{t.u. x } m^3}{\text{min}}$$

The total TER can be a useful measure of toxic pollution of the estuary with the various wastes from the different sources.

After virtually complete mixing of the several effluents and the available dilution water, the average toxicity concentration in the estuary is not likely to be high enough to be measurable by acute toxicity bioassay. For several reasons, it cannot be reliably computed from data on the initial toxicity and flow rates of the effluents and the degree of their dilution. The true value is likely to be less than the computed value, mainly because of natural self-purification processes in the receiving waters; these processes result in progressive loss or degradation of most toxic pollutants at varying rates. The true toxicity concentration can also be greater than the computed value, however, because of synergism of persistent toxicants from the different sources or the production or liberation in the receiving waters of toxic substances not present in the same form in the effluents. Disregarding all of these possible sources of error, the computation in question can be made simply by dividing the total TER value by the total volume of wastewaters and unpolluted dilution waters entering the estuary per minute. For example, if the daily average inflow of dilution water entering the estuary (including tidal exchange) is about 3 000 m^3/min, the computed toxicity concentration = 330/3 330 = 0.10 t.u. The estimation of the toxicity concentration at any given point in the estuary where mixing of the effluents and dilution water is not complete (again assuming persistence of all toxicants introduced, etc.) is more involved and difficult and cannot be adequately considered here.

In summing the TER values for a number of different effluents, the assumption is made that the toxic effects of all the effluents are strictly additive. This assumption is not always valid. However, in the absence of evidence to the contrary, the assumption of simply additive joint action of the different, combined toxicants is justifiable, for it is supported by a considerable amount of empirical data. For example, such diverse toxicants as phenol, copper, and ammonia have been found to interact in a nearly additive manner in producing lethal effects on freshwater fish.

It has been indicated already that not all of the fundamentally different approaches to waste disposal control that have been favoured by some authorities require attention to the condition of receiving waters. Uniform standards of wastewater quality or quantity that are independent of the assimilative capacity of receiving waters have some advantages, such as ease of enforcement, but there is no sound biological justification for such effluent standards. Whether entirely arbitrary or based on considerations such as technological and economic feasibility of compliance the adoption of standards limiting the acute toxicity of effluents or limiting the toxicity emission rates without regard to dilution rates is certainly a questionable regulatory practice. It can be inadvisable for a number of reasons.

The standards may not provide adequate protection for aquatic life against sub-lethal injury or chronic toxicity when effluents which conform with the standards receive too little dilution. Furthermore, the uniform effluent standards may discourage location of new industrial plants where the environmental impact of the waste discharges would be minimal. While supposedly providing for even-handed treatment of competing industrial concerns, they can actually be inequitable and can encourage industry to put other considerations ahead of those pertaining to environmental protection when sites for new plants are selected. Overtreatment of wastes to meet unnecessarily restrictive standards may involve not only unnecessary monetary costs but also a waste of natural resources, such as fossil fuels required for the generation of necessary power, and undue degradation of the total environment, such as increased pollution of air or land. Such overprotection of the aquatic environment thus can be ecologically, as well as economically, unsound. Standards simply limiting uniformly (without adjustment for differences in amounts of dilution) the acute toxicity of the effluents, i.e. their toxicity concentrations, and not the toxicity emission rates, can be especially incompatible with natural resource conservation. They tend to discourage frugal use of water by industry and to interfere with maximum efficiency of waste treatment. Treatment of a wastewater is usually most effective in reducing the quantities of pollutants discharged when the wastewater is relatively concentrated and its volume is relatively small.

REFERENCE

California Water Resources Control Board, 1972. Water quality control plan for ocean waters of California. State of California, Water Resources Control Board, 18 p.

Appendix 3

IDENTIFICATION OF RESEARCH NEEDS

The following list is by no means exhaustive. However, it defines areas of research where obvious gaps exist which, in the view of the Working Party, hamper progress.

(a) The results of published bioassays should be assembled and summarized as to pollutant, toxicity, organism, test period, type of test and geographical region.

The number of people, laboratories and countries conducting bioassays is expanding, but there has been little effort to correlate ensuing results. This would be a difficult task, which is complicated by the large number of species used and types of pollutants tested and differences in the duration of the tests, the experimental conditions, etc. However, some synthesis is required on a global scale. It should be updated at intervals.

(b) There should be more comparisons of the results of 96 hour and threshold lethality tests with those of long-term sub-lethal toxicity tests such as tests of reproductive success, physiological and biochemical responses, etc.

Such comparisons are useful to assess the predictive value of application factors in the calculation of "safe" levels. They are also necessary to assess the significance of concentrations of pollutants which have been shown to have sub-lethal effects, in relation to those concentrations causing death.

(c) There is strong need for nomination of additional marine fish species which are suitable for sub-lethal toxicity testing through a complete life cycle, i.e. from egg to the next generation of eggs.

Ideally, such species should be small, with a life cycle of only a few months, and easily amenable to life in aquaria. Preferably, the species should have a wide geographical range, although this is not essential. Exotic freshwater species have gained wide acceptance for laboratory testing outside their natural range, and they have proved to be reasonably predictive of effects on native fish. The present lack of generally accepted marine species is a distinct handicap to pollution testing.

(d) There is a need to develop rapid "short-cut" sub-lethal tests for marine organisms.

Such tests might be, for example, "coughing" in fish or regeneration of byssal threads in mussels, or change in ciliary beating in invertebrates. However, the research must also relate the effects measured by such rapid tests to sub-lethal effects which are meaningful in the life of whole organisms, influencing populations, communities and production rates.

(e) Intercalibration between laboratories should be tested by standardized, parallel bioassays, and good reference toxicants should be developed.

Intercalibration experiments are necessary to compare results from one laboratory with those from another. This type of experiment is of

particular value in training and checking laboratories and personnel. Careful planning of the intercalibration experiment is essential if the results are to be valid, and use of one or more standard toxicants is a basic requirement.

Standard toxicants would also be of value as reference chemicals against which the results of toxicity tests with other pollutants can be compared. This would expose deficiencies in test techniques or abnormal sensitivity of the test organisms. Although several chemicals have been used as reference toxicants, no wholly satisfactory substance has been found.

(f) Improved chemical procedures are necessary for the identification of toxic "chemical species" for both research and monitoring programmes.

The chemical analytical techniques used for some important pollutants do not measure the components which are toxic to aquatic organisms. For example, in monitoring programmes, measurements are usually made of total dissolved copper, of which only a part may be in highly toxic ionic form and the remainder may be complexed with soluble organic compounds in colloidal suspension. Data from such analyses do not give a true assessment of the effect which this metal may have on aquatic organisms. Collaboration between chemists and biologists is essential for the development of relevant analytical techniques.

M - 45
ISBN 92 - 5 - 100300 - 9